# Recovery Plan for

### **Zebra Chip of Potato**

# Caused by 'Candidatus Liberibacter solanacearum' December 2014

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to ensure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks can maintain a reasonable level of crop production.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that can address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

#### **Executive Summary**

The US potato industry had farm-level sales of nearly \$4 billion in 2013 and constitutes a vital segment of American agriculture. However, the economic sustainability of this industry is threatened by an emerging disease named Zebra Chip (ZC). Zebra chip is putatively caused by a fastidious, phloem-limited bacterium, 'Candidatus Liberibacter solanacearum' (Lso), which is vectored by the potato psyllid (Bactericera cockerelli). The disease was named for the characteristic striping and discoloration in potato chips produced from infected tubers, but it affects all market classes of potato, by reducing both yield and quality. ZC was first reported in the US from South Texas in 2000, but now has spread to other major production regions across the western US and is widespread throughout Mexico, Central America and New Zealand.

Genetic variability has been identified in populations of *B. cockerelli*. At least four potato psyllid haplotypes exist in the US, correlating to the Southwestern, Central, Western, and Northwestern US. Furthermore, potato psyllids collected from northern production areas can withstand colder temperatures for longer periods of time than psyllids collected from Texas, and psyllids of the Northwest haplotype have been found overwintering on bittersweet nightshade (*Solanum dulcamara*) in Idaho, Oregon and Washington states. These findings clearly indicate the existence of multiple, unique populations of potato psyllid in the US and demonstrate that local, overwintering populations could be responsible for disease outbreaks, as opposed to populations migrating from Mexico.

Genetic variability also exists in Lso and two haplotypes, designated A and B, have been identified in the US. Both haplotypes are common in Central America, Mexico and the southern and central US, but only haplotype A has been identified in the Pacific Northwest and New Zealand. Analysis of Lso from diseased potatoes, collected in the US between 2004 and 2014, indicates that a shift from haplotype B to haplotype A may be occurring and this could be significant in that preliminary studies indicate haplotype B is more virulent. Furthermore, there appears to be significant interactions between vector and pathogen haplotypes.

Management of ZC primarily is achieved by frequent application of insecticides for vector control. Because of the potential for huge economic losses, the action threshold for insecticide application for most growers is a single psyllid. Six to twelve applications per year are common, including an in-furrow treatment at planting, and this has resulted in resistance to certain classes of insecticides in some psyllid populations. Currently no cultivars with genetic resistance to either *B. cockerelli* or Lso are commercially available. However, breeding germplasm, screened for resistance to *B. cockerelli* and Lso, has exhibited reduced psyllid feeding and resting, suggesting resistance to the vector, and several clones have displayed significantly reduced Lso infection.

#### **Recommended Actions:**

- Develop improved methods to detect and quantify Lso in the potato psyllid and in plant tissue, and to monitor psyllid numbers in grower's fields.
- Determine factors that impact frequency of Lso in potato psyllid populations and use that information to develop a risk-assessment model.
- Develop an action threshold for application of insecticides for control of the potato psyllid.
- Identify alternatives to high frequency insecticide applications for management of ZC, including biological control and genetic resistance.
- Develop programs and publications (including versions in Spanish) to enhance grower education in all aspects of ZC, but especially with regard to dangers associated with incorrect insecticide applications and late season/post-harvest disease management.
- Educate and train extension personnel, growers and crop advisors in the symptomatology and detection of ZC.

# *'Candidatus* Liberibacter solanacearum' Putative Causal Agent for Zebra Chip of Potato

**Contributing authors:** Charlie Rush\* and Fekede Workneh - Texas A&M AgriLife Research; Neil Gudmestad – North Dakota State University; Don Henne – Monsanto, Maui, HI; Chris McIntosh and Arash Rashed – University of Idaho; Stuart Reitz – Oregon State University; John Trumble – University of California, Riverside

\* Chair and corresponding author: Charlie Rush (crush@ag.tamu.edu)

#### I. Introduction

In 2000, a new disease of potatoes called Zebra Chip (ZC) was first reported in commercial potato fields near Weslaco, TX, in the lower Rio Grande Valley. The disease was named for the characteristic striping and discoloration in potato chips produced from infected tubers, but ZC affects all market classes of potatoes, from processing to fresh market. When ZC was first identified, it was considered a "south Texas" problem and generated little concern, even though the cause of the disease and how it spread was unknown. However, the disease has since been reported in every major potato production region of the western US, including seed production areas in Colorado, Nebraska and Wyoming. The presence of ZC in the US also has had international implications. It has led to quarantines imposed by Korea and Costa Rica and raised concerns among a number of other countries about the safety and quality of potatoes from the US.

ZC has been economically important since the mid-1990s in the fresh market potato producing areas of Mexico, particularly in the states of Coahuila and Nuevo Leon in northeastern Mexico. This area borders the winter potato production area in Texas where ZC first was found in the U.S. In 2004 and 2005, ZC was responsible for millions of dollars in losses to Texas producers and processors (Rosson, et al. 2006; Goolsby, et al. 2007). The disease spread, and in 2006, 2007 and 2008 severe losses were reported in west Texas and the Texas High Plains, Kansas and California. In 2011, it was first reported, in the Pacific Northwest (PNW), where nearly 60% of the entire US potato crop is produced (Wen, et. al., 2009, Lin and Gudmestad, 2013).

When ZC was first discovered in the US in 2000, nothing was known about disease etiology or epidemiology. However, results from a number of studies supported the hypothesis that ZC was caused by a plant pathogen. Tubers from ZC-affected plants could produce symptomatic seedlings (Henne, et al., 2010), and healthy plants grafted to diseased plants became symptomatic (Crosslin, et al., 2009; Secor, et al., 2009). One of the first pathogens to be associated with ZC was a 'stolbur-like' phytoplasma named 'Candidatus Phytoplasma americanum' (Lee, et al., 2006; Secor, et al., 2006). However, it was later determined that this organism was not the cause of ZC (Wen, et al., 2009).

In 2008, two labs independently found an association between 'Candidatus Liberibacter' and two distinct diseases of potato; psyllid yellows and ZC (Hansen, et al., 2008; Liefting et al., 2008a). The first group found a 'Ca. Liberibacter' species that infected B. cockerelli, and its

solanaceous hosts tomato and potato, where it caused psyllid yellows (Hansen, et al., 2008). This bacterium was found in psyllid populations from Texas and California as well as in diseased potato and tomato plants. Based on a phylogenetic analysis using 16s rRNA, the new bacterium nested within the genus 'Candidatus Liberibacter' and was tentatively designated as 'Candidatus Liberibacter psyllaurous' (CLp) (Hansen, et al., 2008). Results from PCR screening of eggs, and egg transfer experiments, indicated that CLp was vertically and horizontally transmitted through *B. cockerelli* life stages (Figure 1).



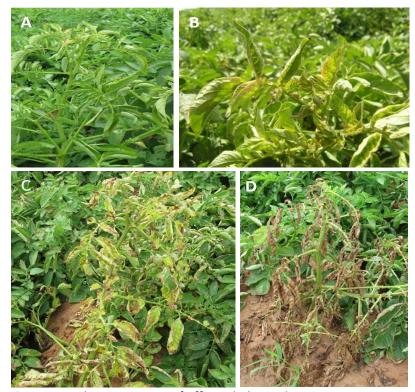
Figure. 1. An adult potato psyllid (A), nymph (B), and eggs (C) on potato leaves.

A second research group found a 'Ca. Liberibacter 'sp. associated with a new disease of Capsicum sp. in New Zealand, causing symptoms in potato similar to ZC (Liefting, et al., 2008b; 2009). The proposed name for this organism was 'Candidatus Liberibacter solanacearum' (Lso) (Liefting, et al., 2009b), as it infected a number of solanaceous hosts (Liefting, et al., 2008a; 2008b; 2009a; 2009b). It is now apparent that 'Candidatus Liberibacter solanacearum' and 'Candidatus Liberibacter psyllaurous' do not represent different species, since their 16S rRNA gene sequence shows a 99.95% similarity (Lin, et al, 2008). Nonetheless, these independent research discoveries strongly suggested that ZC was caused by a 'Ca. Liberibacter' sp., and that it was vectored by the potato/tomato psyllid. Using available PCR technology, Lso was confirmed in ZC affected potato plants from Texas (Abad, et al., 2009, Secor, et al., 2009), California (Crosslin and Munyaneza, 2009) and Mexico (Munyaneza, et al., 2009). Later, using an array of PCR primers, 'Ca. Liberibacter' sp. were found associated with ZC affected plants collected from Texas in 2005-2007 (Wen et al., 2009). The similarity among all 'isolates' of Lso was >99% (Wen, et al., 2009), indicating very little variability, which agreed with studies of the entire 16S rRNA genome (Lin, et al., 2009). Wen et. al. (2009) also confirmed the presence of Lso in infected potatoes collected from Colorado, Kansas and Nebraska in 2008, and in seed potato tubers produced from Wyoming in 2007. Based on these data, and a series of experiments that implicated the potato psyllid with ZC (Munyaneza, et al., 2007a; 2007b) it was clear that ZC was spreading, and had potential to develop wherever B. cockerelli was found.

## **II. Signs and Symptoms**

Potato plants are susceptible to infection by Lso at all stages of development and transmission of Lso by a bacteriliferous potato psyllid can occur in hours. However, it takes

approximately three to four weeks after infection for the initial foliar symptoms of ZC to appear. For this reason, plus the fact that most farmers apply infurrow insecticide applications at planting that protect the plant for a couple of weeks after emergence, symptoms of ZC are seldom observed in grower's fields before flowering. Potato plants infected by Lso exhibit a range of foliar symptoms (Figure 2). Emerging leaves at stem terminals exhibit a slight upward curling and purpling or chlorosis, and not all stems from the same plant exhibit symptoms. However, within week following initial symptom appearance, most leaves on the plant will exhibit severe leaf



**Figure. 2.** Foliar symptoms of affected plants showing progressive intensity in severity from leaf cupping (A) to plant death (D).

curling and chlorosis. Internodes near stem terminals become shortened and may exhibit a zigzag growth habit and aerial tubers frequently develop on stems near the soil surface (Figure 3). Leaf and stem necrosis develops, vines collapse and plants die. Symptoms develop rapidly and



**Figure. 3**. Symptoms of affected plants showing excessive proliferation of shoots and buds on the stem (A), aerial tubers at lower stem (B), and rotting of affected lower stem (C). can progress from asymptomatic to plant death in three weeks.

Typically, infected, symptomatic plants first appear around the periphery of the field, resulting in a definite edge effect (Workneh, et al., 2012) in which clusters of diseased plants develop. In the absence of adequate vector control, psyllids populations increase rapidly, resulting in widespread disease incidence across fields, but even in well managed fields clusters of diseased plants may develop randomly (Figure 4).

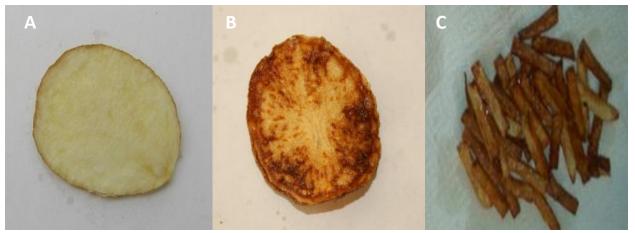


Figure. 4. A cluster of yellowing and dead plants (A) and a field showing extensive damage by the disease

Although foliar symptoms can be confused with those caused by herbicide damage, fungal root rots or other diseases, tuber symptoms are relatively diagnostic for ZC. Tuber symptoms of ZC begin to first appear approximately two to three weeks following plant infection and precede the onset of foliar symptoms (Rashed, et al., 2013). Stolons become necrotic and collapse and the stolon attachment region on freshly dug tubers typically exhibits a pinkish-brown discoloration. Infected tubers are often smaller than healthy tubers, but this depends on developmental stage of the plant when infected. When an infected tuber is sliced, internal symptoms include brown discoloration of the vascular ring and necrotic flecking or streaking of the medullary ray tissues (Figure 5). In the early stages of plant infection, not every tuber from an infected plant will exhibit symptoms and symptoms in infected tubers will be worse at the stolon attachment end. However, eventually most tubers from an infected plant will become symptomatic and symptoms will be visible throughout the tuber. Plants infected early in the season exhibit the most severe symptoms with concomitant reductions in tuber yield and quality, and often die. When such tubers are sliced and fried, the resulting chips or fries are dark and of unacceptable quality (Figure 6). Tubers from plants infected late in the season may be asymptomatic and test negative for Lso at harvest, thereby resulting in a significant underestimation of disease incidence. These tubers may be placed into storage with no symptoms of ZC and undetectable levels of Lso, but depending



**Figure. 5.** Symptoms of affected tubers showing pink color at stolon attachment "pink belly button" (A), browning of the medullary rays on a sliced tuber (B), and a healthy and two symptomatic tubers



**Figure. 6.** Fried chips from a healthy tuber (A) and a ZC-symptomatic tuber (B), and French fries processed from ZC-symptomatic tubers (C).

on pulp temperature at harvest and the length of time it takes to cool tubers down to their set temperatures, typically around 6 C, Lso and disease symptoms can continue to develop in storage. Likewise, if plants are infected within a week before vine kill and tubers are left in the soil for a few weeks before harvest, typical symptoms of ZC can develop in the tubers and when processed they will be of unacceptable quality (Rush, et al., 2014). However, even tubers that are infected but asymptomatic can produce unacceptable chips or fries when processed. This is a result of the rapid changes in tuber physiology associated with infection by Lso, which interfere with carbohydrate metabolism (Gao, et al., 2009), amino acid composition (Yang et al., 2011; Wallis et al., 2012) and phenolic contents (Navarre, et al., 2009; Wallis, et al., 2012).

**Symptoms associated with infected seed potatoes.** Symptoms of ZC typically first appear in grower's fields after flowering but if seed potatoes are infected by Lso, systemically infected, symptomatic plants may appear two to three weeks after planting. When seed potatoes infected by Lso are planted, emergence is typically very low and not all seedlings will be infected by Lso (Henne, et al., 2010; Rashed, et al., 2014a). However, some will exhibit symptoms of systemic infection, i.e., severe stunting, leaf curling and chlorosis. Such plants are quickly overgrown by



**Figure. 7.** Seedlings emerging from seeds infected with the bacterium showing stunting and curled leaves (A) and wilting (B).

surrounding healthy plants and usually die within a few weeks and for these reasons are likely unimportant in the epidemiology of ZC (Figure 7).

#### III. Spread and Risk Map

The potato psyllid is the only insect vector associated with ZC, but for many years it has been widely regarded as a destructive insect pest of potatoes in the western hemisphere. By the 1920s and 1930s, the potato psyllid was causing widespread damage to potatoes in the southwestern United States, giving rise to the description of a new disease of potato known as 'psyllid yellows' (Richards, 1928; Binkley, 1929; Richards and Blood, 1933). Although found throughout a broad geographical range in the western U.S. and southern Canada, the potato psyllid is rarely encountered east of the 22-inch rainfall isoline (Goolsby, et al. 2012).

Although there is some year-year variability in psyllid population dynamics, potato psyllid activity in the central U.S. is characterized by a bimodal population trend, with psyllids in south Texas (LRGV and Pearsall) comprising the winter season population cycle (November-April), and the Colorado/Nebraska location comprising the summer population cycle (May-September). Psyllid populations in the Olton/Dalhart region of Texas have no distinct pattern. This region is intermediate geographically between the south Texas and Colorado/Nebraska populations, and may be a bridge population between the southern and northern central U.S. populations. Potato psyllids are first detected in the LRGV at the end of November-early December, slightly later in the Pearsall area. Adult activity peaks during the latter half of March and continues through mid-April. Activity ceases by late May-early June and no activity is detected again until the following November. In both of these areas, potato psyllids are actively breeding from December to March.

It has long been theorized that potato psyllids migrate long distances from their overwintering breeding sites in northern Mexico and southern US regions to production areas as far north as the Canadian border. Psyllid populations are likely driven, both locally and regionally, by environmental factors such as temperature, rainfall, host quality, etc. Years with mild winters occurring over large geographic areas (such as 2011-2012) allow winter breeding psyllids to build up larger populations than usual, which in turn could trigger migration. Wallis (1946) listed 6 reasons that provided support for the migration hypothesis, and while these were convincing at the time, recent studies have provided results that also support the existence of local, established populations, within western potato production regions.

Studies conducted under controlled conditions, demonstrated that potato psyllids are able to survive for several hours when exposed to sub-freezing temperatures (Henne, et al., 2010; Whipple, et al., 2012), and that psyllids collected from Nebraska exhibited greater cold tolerance than psyllids collected from Texas (Whipple, et al., 2012). These studies provided an indication of genetic variability among psyllid populations, with regard to temperature tolerance. Later, work by Swisher, et al. (2012), using high resolution melting (HRM) analysis, confirmed the existence of four haplotype populations that corresponded roughly to large geographic regions, i.e., western, central, southwestern and northwestern. There is considerable overlap among these populations but the northwestern haplotype has only been identified in the PNW. Finally, Jensen (2012) reported the overwinter survival of potato psyllids on bittersweet nightshade (*Solanum dulcamara*), in Idaho and overwintering subsequently was confirmed in Oregon and Washington State. The results of these studies clearly indicate the existence of multiple, unique populations

of potato psyllid in the US. While these studies do not preclude the possibility of long distance psyllid migrations, they do demonstrate that local populations exist, even in northern production areas, and may impact timing and incidence of ZC.

#### IV. Detection and Identification

Pathogen Detection. The early detection of Lso in affected potato plants, before the onset of symptom development, and the detection of the pathogen in its insect vector, is essential in studying ZC disease epidemiology and in the development of effective disease management strategies. Since ZC is caused by a prokaryotic pathogen, most of the PCR methods developed utilize 16S and 23S ribosomal DNA sequences (Hansen, et al. 2008; Li, et al. 2009; Liefting, et al. 2008a, 2008b; Ravindran, et al. 2011, 2012; Secor, et al. 2009; Wen, et al. 2009). While a number of forward primers have been empirically designed based on the 16S rRNA sequence of Lso (NCBI GenBank accession number EU980389 (Li, et al. 2009, Liefting, et al., 2009a and 2009b; Wen, et al., 2009a), the reverse primer most commonly used for the PCR reaction is the Liberibacter-universal primer Ol2c (Jagoueix, et al. 1996). As a result, some of the primers developed to detect Lso also amplify some strains of Las and Laf (Ravindran, et al. 2012; Wen, et al. 2009). Fortunately, all of the PCR primers developed to date are specific for the "Candidatus Liberibacter" sp. associated with the zebra complex and do not detect other prokaryotic plant pathogens that affect potato (Wen, et al. 2009).

The 16S rDNA gene of Lso has also been used in the development six primers for use in a loop-mediated isothermal amplification procedure (LAMP) for the PCR detection of the ZC bacterium in potato plants and its vector, *B. cockerelli* (Ravindran, et al. 2012). Although the procedure proved to not be as sensitive as quantitative real-time PCR (QPCR), it was as sensitive as conventional PCR and has the distinct advantage in that the method does not require a thermocycler for amplification or agarose gel electrophoresis to visualize the results making it potentially very useful for use in the field.

More recently, PCR primers have been designed using intergenic regions of the16s and 23s rDNA genes and a conserved bacterial housekeeping gene, adenylate kinase (Ravindran, et al. 2011). These PCR primers have been demonstrated to be more sensitive than previously published primers (Ravindran, et al. 2011). Genomic DNA-based PCR primers have been developed also to facilitate detection of Lso in the potato psyllid and have been found to be valuable in determining the frequency of the bacterium in its vector (Crosslin, et al. 2011).

A few studies have investigated the effectiveness of detecting Lso in infected potato plant parts. In one study, the overall efficiency of the current PCR technology associated with the detection of true positives was demonstrated to be as low as 60% (Wen, et al. 2009). Detection efficiencies have been generally determined to be higher for below ground portions of plants, such as stolons, roots, and tubers, as opposed to leaves, leaf petioles and above ground stems (Li, et al. 2009; Wen, et al. 2009). The presence of PCR inhibitors in tuber tissues can be problematic, however, and caution needs to be exercised when using tuber tissue to detect Lso infections (Wen, et al. 2009).

Molecular methods of pathogen detection can only be efficient when using high quality DNA. Thus, it is important to verify that the DNA is of sufficient quality to be amplified in a PCR reaction. When PCR assays utilize only a single set of primers to detect the pathogen, a negative

result does not distinguish between the absence of the target, the presence of PCR inhibitors, or poor DNA quality. To overcome this issue, a multiplex PCR platform was designed that combines pathogen specific primers with an internal control utilizing a plant-based DNA target. Two such multiplex PCR methods have been developed to detect Lso in plants, one utilizing a potato  $\beta$ -tubulin gene sequence (Wen, et al. 2009) in a conventional PCR format, the other using a plant mitochondrial cytochrome oxidase gene sequence in a real-time PCR format (Li, et al. 2006, 2009).

Despite recent improvements in PCR detection technology (Crosslin, et al. 2011; Ravindran, et al. 2011), there is still a need for improvement of PCR technology. For example, the development of a multiplex PCR format that can distinguish Lso haplotypes (Lin, et al. 2012; Nelson, et al. 2011) while simultaneously determining potato psyllid biotypes (Jackson, et al. 2009; Swisher, et al. 2012), would be very useful in epidemiological studies. Currently, Lso haplotypes are distinguished using conventional PCR primers based on genomic DNA-based gene sequences (Wen, et al. 2013) and psyllid haplotypes can be distinguished using high resolution melting curve analysis (Swisher, et al. 2012).

**Vector Detection.** Over the past few years significant advancements have been made in understanding how psyllid populations can be measured in potato fields. Studies have revealed the most efficient plant sampling unit, the spatial dispersion of psyllids in potato fields, and a simple and effective binomial sequential sampling plan for psyllids has been developed (Butler and Trumble, 2012a). However, important questions still remain. The action thresholds currently in use were designed to allow growers to choose the level of risk they are willing to accept. These treatment thresholds do not provide estimates of yield losses that may occur. One confounding factor is that not all psyllids are infected with the Lso pathogen. While uninfected psyllids still cause plant loss (aka psyllid yellows), psyllids that transmit Lso can substantially reduce tuber quality even late in the season when visual symptoms are not evident (Rashed, et al., 2013; Rashed, et al., 2014b, 2014c).

A potato psyllid monitoring program has been established and is comprised of a network of locations in multiple states (and one Canadian Province): Texas, Nebraska, Kansas, Colorado, North Dakota, Minnesota, Wisconsin, and Manitoba, Canada. In Texas, commercial potato fields near Edinburg, Pearsall, Olton, Springlake, and Dalhart are sampled. In other states, locations near Scottsbluff and O'Neill, Nebraska; Alamosa, Fort Morgan, and Wray, Colorado; Garden City, Kansas, and numerous locations in North Dakota, Minnesota and Wisconsin are sampled. Untreated potato plots are also maintained at several locations as close as possible to commercial fields. At each commercial field sampling location, five yellow sticky cards (Pherocon® AM No-Bait Traps, Trécé, Inc., Adair, Oklahoma, USA, Product Code 3306-00) are deployed every 200' along a transect from near the southern edge of fields inward to the center. In the same fields, 100 compound leaves are collected (10 from each of 10 equidistant locations along the field perimeter) and placed in labeled plastic bags. Every week, leaves and sticky traps are shipped to the Weslaco laboratory, where adults on sticky traps were counted using a stereomicroscope, removed and sorted, and then shipped to a diagnostic lab for Lso determination. Leaf samples are processed under a stereomicroscope to determine counts of psyllid eggs, small nymphs (instars 1-3), and large nymphs (instars 4-5). Counts are summarized by state region and compiled into a weekly report that is distributed by email to growers, scientists, industry, and

students interested in the results (approximately 200+ recipients). Reports currently are archived on the SCRI-Zebra Chip website: <a href="http://zebrachipscri.tamu.edu/resources/potato-psyllid-survey-report-archive/">http://zebrachipscri.tamu.edu/resources/potato-psyllid-survey-report-archive/</a>. Preplanting surveys are also performed at some locations to gauge psyllid activity prior to planting, by deploying transects of 20-100 yellow sticky traps.

Although sticky traps are widely used to monitor psyllids in grower fields, there are problems with this technique. It has not proven suitable as a predictor of population densities within plants in California (Prager, et al. 2012), it did not match vacuum samples from plants in Idaho (Wenniger, et al. 2012), and it was not considered as effective as leaf washing in Texas (Seibert, 2011). However, data from sticky traps does have predictive value and are routinely used by growers to time insecticide applications. Data from sticky traps can be used to document initial migrations into specific agricultural regions and the potential exists to increase reliability by modifying color, shape, or height of deployment.

Currently, researchers are investigating attractant lures from plant volatiles, and development of improved molecular and external/visual diagnostics for psyllid haplotypes. These new approaches have potential to be combined with traditional trapping techniques and improve overall detection and quantification of psyllid populations in grower's fields.

#### V. Response

The response to all plant health emergencies is under USDA-APHIS, Plant Protection and Quarantine's authority, under The Plant Protection Act of 2000 (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). 'Candidatus Liberibacter solanacearum' is not listed as a Select Agent and the initial response to ZC in the US, when neither the vector nor pathogen had been identified, primarily was from affected growers, processors and agri-industry personnel. However, USDA-APHIS ultimately was involved in confirming Lso in solanaceous crops in the United States following discovery of the bacterium in New Zealand (Abad, et al., 2009). USDA-APHIS is also involved in addressing issues related to international trade of Lso-affected agricultural commodities, including potato.

The ultimate authority for confirming a diagnosis of the disease rests with the Plant Protection and Quarantine (PPQ) division of APHIS: <a href="http://www.aphis.usda.gov/ppq">http://www.aphis.usda.gov/ppq</a>. After a detection of ZC is confirmed by a USDA, APHIS, PPQ recognized authority, APHIS, in cooperation with the State Department of Agriculture, is responsible for the response. In most cases, large numbers of suspect plant samples will be collected by growers, processors and agri-industry personnel for testing. Detailed protocols for PCR diagnostics, specific to Lso, are available and samples can be tested by labs associated with the National Plant Disease Network in each state. Furthermore, there are several researchers around the country who have extensive expertise in diagnosing ZC and their labs are available to test suspect plant samples in instances of a new outbreak of ZC (N. Gudmestad, NDSU, Fargo; A. Karasev, U of Idaho, Moscow; H. Lin, USDA-ARS, Parlier, CA; J. Munyaneza, USDA-ARS, Wapato, WA; C. Rush, Texas A&M AgriLife Research, Amarillo).

Once ZC has been identified and officially confirmed in a new production region, immediate efforts to educate growers, processors, other stakeholders and the public in general should be initiated to avoid raising unnecessary concern. It should be made clear that ZC is a quality issue for growers and does not constitute a health risk for the general public. Considerable published information on ZC is available and should be provided to regional news

media services, and state Extension personnel should initiate educational meetings and training programs with growers, crop consultants and industry personnel. At the same time, disease surveys should be initiated to determine the extent of the disease. Survey teams should conduct delimiting surveys in the area using trace back and trace forward information and with various appropriate stratified delimiting sampling schemes for surveys in the area of detection. If the disease outbreak is widespread, impacting large areas, this especially will be important in attempting to identify the source of bacteriliferous psyllids. Other studies and surveys should be conducted to determine if a widespread disease outbreak is due to unusual environmental conditions or changes in grower activities. In the case of seed potato production, it is important, as part of the response, to control the movement of diseased host tissues of infested counties since this may serve as an avenue to introduce ZC into other production areas. Agrichemical companies, aerial applicators and others potentially involved in disease management should be made aware of the potential for increased demand for their products and services.

#### **VI. USDA Pathogen Permits and Regulations**

USDA-APHIS-PPQ permit and registration requirements for plant diseases and laboratories fall under the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status, that are shipped interstate and also requires that the receiving laboratory have a permit: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ permit services at (301) 734-8758. This procedure may limit early detection of ZC since it complicates sending and receiving samples for identification (confirmation), although there are diagnostic laboratories in every state and all NPDN laboratories have APHIS permits to handle "unknown" diagnostic samples. Concerted efforts to educate first detectors will insure the proper handling and identification of potential Lso-infected plant material in the event that a suspect high consequence sample is found.

#### VII. Economic Impact and Compensation

The spread of Zebra Chip (ZC) disease creates economic vulnerabilities for potato producers worldwide and threatens international food security. Potatoes are the fourth most consumed food crop in the world and have a water utilization efficiency that is up to seven times greater than cereal crops (CIP, 2010). As a result, potatoes produce more food per unit of water than any other major food crop. In the US, potatoes are one of the most commonly consumed vegetables offering some of the lowest nutrient costs per penny of investment when obtaining potassium, fiber, and vitamin C (USDA-ERS, 2013; Drewnowski and Rehm, 2013). They are also a high value crop for producers and offer potential for significant economic profit in the absence of disease. However, if incidence of defects (this includes ZC) in a potato crop exceeds a predetermined, contract-specified level the entire crop will be rejected. In such instances, growers not only lose the negotiated sale price of the crop, but they also incur additional losses associated with disposal of the diseased crop.

**Impact of ZC on Trade.** The superior quality and availability of US potatoes has led to steady gains in worldwide market share. According to the United States Potato Board (2013) total US potato and potato product exports have grown 133 percent in value and 79 percent in volume in the ten year period of 2003 to 2013. In fiscal year 2013, the estimated total value of all US potato and potato product exports was over \$1.5 billion. ZC infection threatens the quality attributes that make US potatoes attractive to export markets and jeopardizes international market expansion efforts by US producers.

Cost of ZC in the Southwest. The current ZC control plan is expensive and pesticide intensive. Greenway, et al. (2013) documented insecticide applications in commercial potato fields in Texas, Kansas, and Nebraska from 2009 to 2013. Texas growers used a total of 23 different insecticides to manage ZC during the five year timeframe. Kansas and Nebraska growers used a total of 27 insecticides for ZC management. The five-year average number of insecticide applications per season for control of ZC in Texas was 8.9; in Kansas and Nebraska the five year average number of applications was 9.4. The five year average cost was about \$704 per hectare in all Texas locations. The range of costs in Texas fluctuated from a low of \$363 per hectare to a high of \$1,467 per hectare. The five year average cost per hectare in all Kansas locations was \$699 and the five year average cost in all Nebraska locations was \$563 per hectare. The range of costs in Kansas and Nebraska varied widely over the five year timeframe from a low of \$76 per hectare to a high of \$1,272 per hectare.

**ZC** in the Pacific Northwest. The source of psyllid colonization in the Pacific Northwest remains unknown; growers are unable to pinpoint psyllid hotspots, and cannot predict the number of psyllids or the timing of their arrival (Horton, et al. 2014). The many uncertainties regarding psyllid pressure creates an inability to predict the exact number of insecticide applications needed to protect the crop in various regions adding significant complications to the pre-season contract negotiation process.

Idaho is the largest potato producing state responsible for over 30 percent of US production (USDA-NASS, 2014). About 39 percent of the Idaho crop is diverted to the frozen category of the processed potato market. Pre-season contracts function to guarantee processors access to a stable supply of high quality raw product while helping to reduce the risk of price volatility for growers. Contracts are designed to be a mutually beneficial mechanism for growers and processors, but historical analysis from Idaho highlights the inability of contract prices to keep pace with costs of production (Patterson and Bolotova, 2011).

Contract prices provided by the Southern Idaho Potato Cooperative and University of Idaho costs and return estimates for the Eastern region of Idaho from 2003-2012 highlight shortfalls between increases in contract payout price and increases in cost of production in five of the ten years. Even without ZC Idaho producers have failed to consistently negotiate contract prices that keep pace with costs of production. The uncertain threat posed by psyllid pressure could further jeopardize the ability of growers to negotiate profitable contract prices providing incentive to exit the industry in favor of less risky alternatives.

#### **VIII. Mitigation and Disease Management**

Currently, control of potato psyllids and management of zebra chip is achieved almost entirely through application of insecticides designed to control the psyllid vector. While some of these applications may be tied to information resulting from sampling, most growers apply pesticides on a schedule. When zebra chip (ZC) first became a problem, numerous insecticides were tried. Since then, though a combination of research and experience, a much more refined approach has been developed. This approach uses a substantially reduced set of materials, which are used commonly wherever psyllid control is necessary. Chief among these materials are certain neonicotinoids (imidacloprid, Admire Pro; thiamethoxam, Platinum), EpiMek/Agri-Mek (abamectin), Fulfill (pymetrozine), Beleaf (flonicamid) and either Oberon (spiromesifen) or Movento (spirotetramat). Because they are widely used across all potato production areas, the potential exists for resistance development.

Research has demonstrated that the potato psyllid has developed resistance to insecticides (Liu and Trumble, 2007; Prager, et al., 2013). The psyllid has developed resistance to the neonicotinoid insecticide imidacloprid in parts of Texas, but not in California (Prager et al. 2013). Interestingly, research over the past several years has demonstrated that the psyllids are still susceptible to another neonicotinoid pesticide, thiamethoxam (Platinum). Studies with the closely related Asian citrus psyllid indicate reduced susceptibility to Movento (spirotetramat) developing after only three years of use. The previous studies of resistance in potato psyllid benefited from baseline toxicity data that allowed for comparison among populations and time points. While some mortality data do exist for Movento (spirotetramat) and Epimek (abamactin), these data were collected for adult individuals and Movento is primarily aimed at immature lifestages. Moreover, the studies did not generate the necessary data for determining levels of resistance, and no data exists for the susceptibility of psyllids to either insecticide outside of Texas. Finally, it is recommended that Movento (spirotetramat) be applied twice, no more than 7 days apart. However, the efficacy of a single application versus two applications is unknown and this is critical information in evaluating potential resistance. In order to maintain the efficacy of these potato psyllid insecticides, it is critical to identify if resistance is developing so usage patterns can be modified to extend the useful life of these materials.

Costs and Benefits of New Rotational Strategies. Over the past few years considerable information on the efficacy of various insecticides for controlling potato psyllids and managing the zebra chip disease has been reported. This has resulted in the development of an effective insecticide rotation for management of ZC. Unfortunately, this also means that growers have settled onto rotations consisting of a relatively small number of insecticidal materials. These materials are often expensive and use some materials that are of questionable efficacy. For example, these rotations often include the application of neonicotinoid insecticides of the same resistance class. As stated earlier, resistance has developed to one of neonicotinoid insecticide and resistance may be developing to a second. Additionally, many current insecticide rotations include EpiMek (abamectin), Fulfill (pymetrozine) and Movento (spirotetramat). There are some concerns about Fulfill as it requires feeding to be effective and this may allow transmission of the zebra chip pathogen. Avermectin is off patent and likely to be overused, especially in Mexico where there is a history of over application of low cost insecticides. Movento (spirotetramat) is used in most rotations and there is currently no effective alternative. In order to maintain the

efficacy of insecticide programs and associated materials, it is critical to have a variety of materials to alternate. Further, as new materials such as Torac™ (tolfenpyrad), Benevia™ (cyantraniliprole) and Cyazypyr™ (cyantranilirople) or Verimark™ are registered, it will be important to properly incorporate them into rotations that manage resistance and ensure maximum effectiveness right from the start. In addition, there are a number of potential repellants that may prove useful (Yang, et al. 2010, Diaz-Montano and Trumble, 2012). Field tests of a series of insecticide rotations are therefore desirable. These rotations should be designed to allow comparisons between the current strategy and rotations incorporating newer materials. Reduced use will delay the development of resistance and prolong the efficacy of current insecticides.

**Biological Control.** Relying on insecticides as the sole control option is not always sustainable, and could result in further problems in management systems through resistance, destruction of natural enemies, and environmental contamination (Van Driesche, et al., 2008). The potato psyllid is attacked by a number of natural enemies in North America (Cranshaw, 1994). Generalist predators that attack the potato psyllid include chrysopid larvae (Al-Jabar, 1999) coccinellids (Knowlton 1933), syrphid fly larvae (Knowlton, 1934b) and various Hemiptera such as *Geocoris decoratus* Uhler, *Orius tristicolor* (White), *Anthocoris melanocerus* Reuter, and *Nabis ferus* (L.) (Knowlton, 1934a; Knowlton, 1934b; Knowlton and Allen, 1936; Cranshaw, 1994). Natural enemies also include two parasitoids (*Metaphycus psyllidis* Compere [Hymenoptera: Encyrtidae], and *Tetrastichus triozae* Burks [Hymenoptera: Eulophidae]) (Yang et al 2010). The fungus *Beauvaria bassiana* is known to attack the potato psyllid as well (Al-Jabar, 1999).

The most recent and complete evaluation of the benefit of psyllid natural enemies in potatoes was published for California (Butler and Trumble, 2012b). A wide range of insects and arachnids were shown to be useful, although many of these reduced populations after adult or nymphal transmission would have occurred. However, they do serve to reduce immigration into the fields and minimize survival of those nymphs that escape pesticide applications. Unfortunately, before these can be successfully integrated into current pest management strategies, such as the previously mentioned chemical rotations, more information is needed on the occurrence and potential effectiveness in other key potato production areas. In addition, there are minimal data on the effects of the key compounds currently used in psyllid control on the most common biological control agents. Essentially no information is available on the impact of the beneficial insects on weeds and other psyllid hosts that contribute to immigration into potato crops. These critical data are a priority for maximizing the benefits that natural control agents may be able to provide.

Breeding for resistance to psyllid/Lso as a component of an IPM approach. Host genetic resistance has potential to be an important component of an IPM program for control of ZC, but to date genetic resistance to ZC has not been identified in commercially available germplasm (Munyaneza, et al., 2011; Anderson, et al., 2013). With no useful resistance to ZC in U.S. and New Zealand potato cultivars, wild *Solanum* species may provide resistance or tolerance that has not been identified in cultivated potato. Unique breeding germplasm derived from the potato species *Solanum etuberosum* and *S. berthaultii* has been shown to impact potato psyllid behavior (Butler, et al., 2011), with antibiosis and antixenosis to potato psyllid having been confirmed

(Diaz-Montano, et al., 2013). Resistance to the potato psyllid vector, as well as possible resistance to Lso (Butler, et al., 2011) can be used by breeders in developing ZC resistant potato cultivars.

#### IX. Research, Education and Extension Priorities

Education of growers and the entire potato industry will be key to managing the ZC problem. In agricultural areas which interface with urban development, homeowners will also need to be informed because the psyllid attacks a wide variety of crop and urban plants (Butler and Trumble, 2012c). Many growers are not aware of the best management strategies and still apply ineffective pesticides or overuse the available useful pesticides. Cooperative Extension personnel will play a key role in educating growers in the best management strategies for ZC. There is a need for scientists with a strongly applied focus to produce literature and educational materials on integrated pest management practices for both growers and homeowners. Specific, high priority, programmatic objectives include:

- Elucidate the specific gene and biochemical interactions associated with pathogen acquisition and transmission, as impacted by different vector populations and pathogen haplotypes, in order to develop effective risk assessment models.
- Investigate the impact of overwintering populations of potato psyllids in the PNW on disease epidemiology and determine whether psyllid biology and vectoring capability is impacted by reservoir hosts, microfauna in the psyllid gut (endosymbionts) or differences among genetically distinct psyllid populations.
- Identify factors responsible for spatial and temporal variations in psyllid abundance/ ZC severity and construct a model (s) using key predictor variables for forecasting the disease.
- Develop new sampling strategies and management tactics that include more sustainable and biological options, and provide an associated economic analysis to assist producers in their decisions of which tactics to implement.
- Identify sources of genetic resistance to the potato psyllid and Lso and incorporate into breeding lines for eventual creation of disease resistant cultivars.
- Determine how late season cultural practices and postharvest storage conditions impact disease development and tuber quality.
- Conduct a comprehensive economic impact analysis for ZC and determine cost-benefit analyses for various management strategies.
- Develop multi-media educational tools and training programs for all aspects of ZC including materials suitable for international distribution.

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#### **Web Resources**

A History in the Making: Potato Zebra Chip Disease Associated with a New Psyllid-borne
Bacterium – A Tale of Striped Potatoes

<u>European and Mediterranean Plant Protection Organization (EPPO) on Candidatus Liberibacter</u> <u>solanacearum</u>

MAF Biosecurity New Zealand: New bacterium affects fresh tomatoes and capsicums

Potatoes New Zealand and Plant & Food Research on Zebra Chip

potatoPRO.com on Zebra Chip Disease

SCRI Zebra Chip of Texas A&M AgriLife Research

The Zebra Chip Project of Texas A&M University

## <u>USDA-ARS: Multi-Pronged Fight Against Zebra Chip Disease in Potatoes</u>

Webcast: Zebra Chip Disease of Potatoes

Zebra Chip